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The Simuliidae (Diptera) of the Republic of São Tomé and Príncipe, including the description of a new species

by

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ABSTRACT

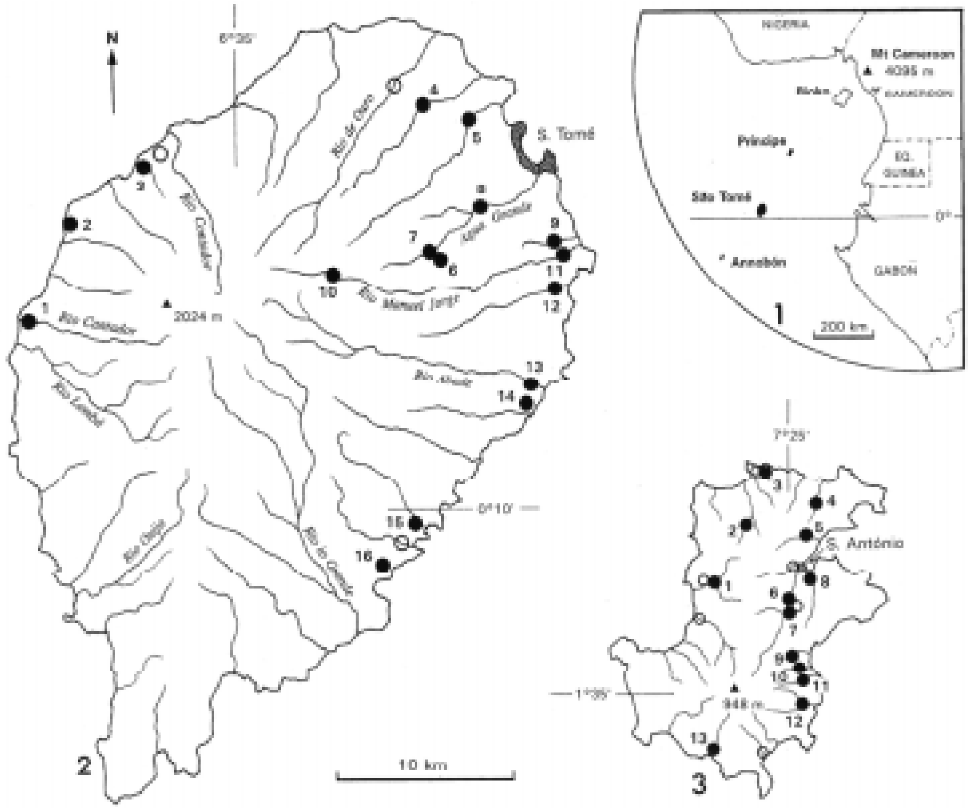
Identifications were made of 1122 simuliid larvae and pupae (including neonate adults) collected from 29 localities during November 1998, on the islands of São Tomé and Príncipe in the Gulf of Guinea. Three species, including one new species, were found on São Tomé. *Simulium* (*Pomeroyellum*) *santomi* sp. n. was particularly abundant in the eastern part of the island, whereas the western part was dominated by *Simulium* (*Anasolen*) *dentulosum* Roubaud. In the north-eastern corner *Simulium* (*Pomeroyellum*) *alcocki* Pomeroy was also found. On Príncipe only *S. dentulosum* was found. Findings are briefly discussed in relation to speciation on oceanic islands and vis-à-vis human onchocerciasis (river blindness).

INTRODUCTION

There are very few published records of Simuliidae from the Gulf of Guinea islands, but there are three reasons why a comprehensive understanding would be valuable. Firstly, because the flora and fauna of these islands have many unique features and hence are of great conservation interest; for example, butterflies show 17 % and 9 % species endemism on São Tomé and Príncipe respectively (Jones 1994). Secondly, because the pattern of variation found along island chains is extremely informative about the biogeography and processes of speciation in general, and as an example of such a chain, the Gulf of Guinea islands are unique in the geography of the Afrotropical Region, and might provide a balance to studies of archipelagos in other zoogeographical regions. Thirdly, in view of the role of man-biting Simuliidae in the transmission of human onchocerciasis (river blindness) in tropical Africa.

The islands of São Tomé and Príncipe constitute the Democratic Republic of São Tomé and Príncipe, one of the smallest African countries. Geomorphologically, the islands are a result of past volcanic eruptions. They form the middle two of a chain of four islands extending into the Gulf of Guinea on a northeast-southwest axis from Mount Cameroon which is also volcanic and is situated on the African mainland (see Fig. 1). The northernmost island of this chain (Bioko) and the southernmost (Annobón), are constituent parts of the Republic of Equatorial Guinea. São Tomé, lying 2 km north of the equator, has an area of 836 km^2 (maximum length 47 km) and is a rugged island in which several peaks attain a height over 1000 m (highest point 2024 m); it is covered by a combination of montane forest and cocoa and coffee plantations. Príncipe lies 150 km northeast of São Tomé and is a much smaller island, its area only 128 km^2 (maximum length 19 km), and its highest peak only 948 m.

The climate of both islands is very similar, with high humidity and temperatures at sea level generally above 25 ^{o}C. Rainfall typically reaches 7000 mm per year on São


N
6°35'
S. Tomé
Rio Contador
2024 m
Rio Manuel Jorge
Rio Abade
0°10'
10 km
2
NIGERIA
Mt Cameroon
4095 m
CAMEROON
Bioko
Principe
EQ. GUINEA
São Tomé
0°
Annobón
GABON
1
200 km
7°25'
S. António
1°35'
948 m
3

Figs 1–3. Maps. 1. Location of the islands of São Tomé and Príncipe in the Gulf of Guinea. 2–3. The islands of São Tomé and Príncipe respectively, showing the major rivers and the localities where immature blackflies were found (numbered sites). River sites where no blackflies were found are indicated with an open circle.

Tomé and 5000 mm on Príncipe, and a rainy season occurs between October and May (Juste & Fa 1994). However, the seasonality is less marked than in continental West Africa, and rain can occur in any month.

MATERIAL AND METHODS

Twenty sites were sampled on São Tomé between 10th and 14th November 1998, and 17 were found to be positive for simuliids (Fig. 2). On the island of Príncipe, 19 sites were sampled between 16th and 19th November 1998, and 13 were found to be positive (Fig. 3). Sites were chosen so as to obtain coverage around the islands at varying altitude, although the southern area of São Tomé is not easily accessible and was not sampled in the survey.

Aquatic stages of Simuliidae (larvae and pupae) were collected from riverine substrates such as leaves, twigs and rocks, from which they were removed with forceps and routinely preserved in 80 % ethanol. Some pupae were reared individually by maintaining them live at room temperature on damp cotton wool in glass tubes. The emergent neonates were killed with ethyl acetate after about 12 hours, and pinned along with their pupal exuviae. Adult biting female simuliids were sought during visits to rivers by exposing the legs of the collector (R. J. Post) and looking to see if flies were attracted.

In total 1122 specimens of larvae and pupae (including neonate adults) were examined. No biting simuliids were found on either island, although diurnal mosquitoes and tabanids were common. All specimens have been returned to the Centro Nacional de Endemias of São Tomé and Príncipe (ST&P) except for vouchers retained in the Natural History Museum, London (BMNH). All specimens were collected by R. J. Post (RJP), except for a single sample collected by Dr Pinheiro de Melo from Ribeira Porco on Príncipe. Simuliidae from the Natural History Museum reference collections, the Instituto de Higiene e Medicina Tropical (IHMT Lisbon, all collected by Prof. Dr A. J. dos Santos Grácio -AJSG) and the Koninklijk Museum voor Midden-Afrika (MRAC, Tervuren, Belgium) were also examined.

List of collecting sites

The following list details the record sites under the numbers used in ‘Material seen’ sections of the species treatments. Geographical co-ordinates of the sites are in north/east sequence.

São Tomé island

1. Rio Cantador south of Santa Catarina, 0°16':6°29'. **2**. Unnamed stream south of Diogo Vaz, 0°18':6°30'. **3**. Água Monte Forte at roadbridge, 0°22':6°41'. **4**. Água Casada 2 km from Santo Amaro, 0°22':6°40'. **5**. Água Palito east of Santo Amaro, 0°22':6°42'. **6**. Água Pequena between Trinidade and Bemfica, 0°18':6°41'. **7**. Água Grande west of Trinidade, 0°18':6°41'. **8**. Água Grande at Bóbó Cativo, 0°19':6°43'. **9**. Água Pété Pété south of Bombom, 0°18':6°45'. **10**. Cascades São Nicolau, 0°17':6°38'. **11**. Rio Manuel Jorge south of Almas, 0°18':6°45'. **12**. Água Clara Dias at Alma Xarife, 0°17':6°45'. **13**. Rio Abade south of Santana, 0°14':6°44'. **14**. Água João Luiz north of Água Izè, 0°13':6°44'. **15**. Rio Angra Toldo east of Angra Toldo, 0°10':6°41'. **16**. Água Moinho north of S. Joao dos Angolares, 0°08':6°39'.

Príncipe island

1. Rio Bacharel below waterfall west of Montalegre, 1°38':7°23'. **2**. Ribeira Izé at Gaspar-Sundi road bridge, 1°40':7°23'. **3**. Água Santa Rita on road to Praia Bom Bom, 1°41':7°25'. **4**. Ribeira Das Voltas west of Paciência, 1°40':7°26'. **5**. Ribeira Faustino south of Ponta Forte, 1°40':7°25'. **6**. Trib. of Rio Papagaio, 1°38':7°25'. **7**. Rio Papagaio above Santo Antonio, 1°38':7°25'. **8**. Ribeira Forca half km upstream from mouth, 1°38':7°26'. **9**. Água da Ponte Grande at roadbridge, 1°36':7°25'. **10**. Ribeira Fria at Ribeira Fria, 1°36':7°26'. **11**. Ribeira Jambela south of Ribeira Frio, 1°35':7°26'. **12**. Rio Chibalá on Infante d’ Henrique road, 1°35':7°25'. **13**. Ribeira Porco, 1°34':7°23'.

KEY TO THE SIMULIIDAE OF SÃO TOMÉ AND PRÍNCIPE

The pupal life stage is initially the most important when investigating blackflies in a little-worked fauna. Hence, to assist any future study, we give the following key by which pupae (and associated larvae) of the three species known in São Tomé and Príncipe can be identified.

1. Gill with 7 or 8 long flexible filaments (in life lying along substrate); cocoon slipper-shaped with slightly strengthened anterior rim (without collar); [associated larva: with pair of conical projections (ventral papillae) on lower surface in front of abdominal attachment organ] 2
– Gill with 14 short stiff branches (not orientated to substrate); cocoon shoe-shaped (sides connected by collar beneath pupal head); [associated larva: without ventral papillae] .... **Simulium** (**Anasolen**) **dentulosum** Roubaud

2. Gill with 7 filaments carried on a common stem on 4 branches (Fig. 13), bifurcating outside-in (2+(2+1))+2; or with 8 gill filaments carried on a common stem in 4 pairs (Fig. 12), bifurcating outside-in: (2+(2+2))+2; [associated larva: upper surface of swollen part of abdomen with small strongly sclerotised narrow fan-like scales (Fig. 19) not easily seen at low-power examination, and rectal organ lobes each with 7–8 secondary lobules] .... **Simulium** (**Pomeroyellum**) **alcocki** Pomeroy
– Gill with 8 filaments carried on a common stem in 4 pairs (Fig. 4), bifurcating outside-in: 2+((2+2)+2); [associated larva: upper surface of swollen part of abdomen with colourless broad fan-shaped setae (Fig. 11), not easily seen at low-power and rectal organs each with 3–4 secondary lobules] ....
Simulium (**Pomeroyellum**) **santomi** Mustapha sp. n.

TAXONOMY

Simulium (**Pomeroyellum**) **santomi** Mustapha sp. n.

(Figs 4–11)

Etymology: The species name is derived from São Tomé, the island from which the species has been collected and described, and where it is probably endemic.

Below is a list of characters that distinguish this new species from *S. alcocki,* followed by a full description**.**

Larvae: Gothic arch-shaped postgenal cleft (Fig. 9), presence of minute broad fan-shaped thoracic setae and abdominal setae (Fig. 11), presence of three or four secondary lobules on the rectal organ and presence of accessory sclerites.

Pupae: Eight pupal gill filaments aligned in mirror image to *S. alcocki* (Fig. 4) and abdominal segment 4 with a pair of ventral spines (Fig. 6).

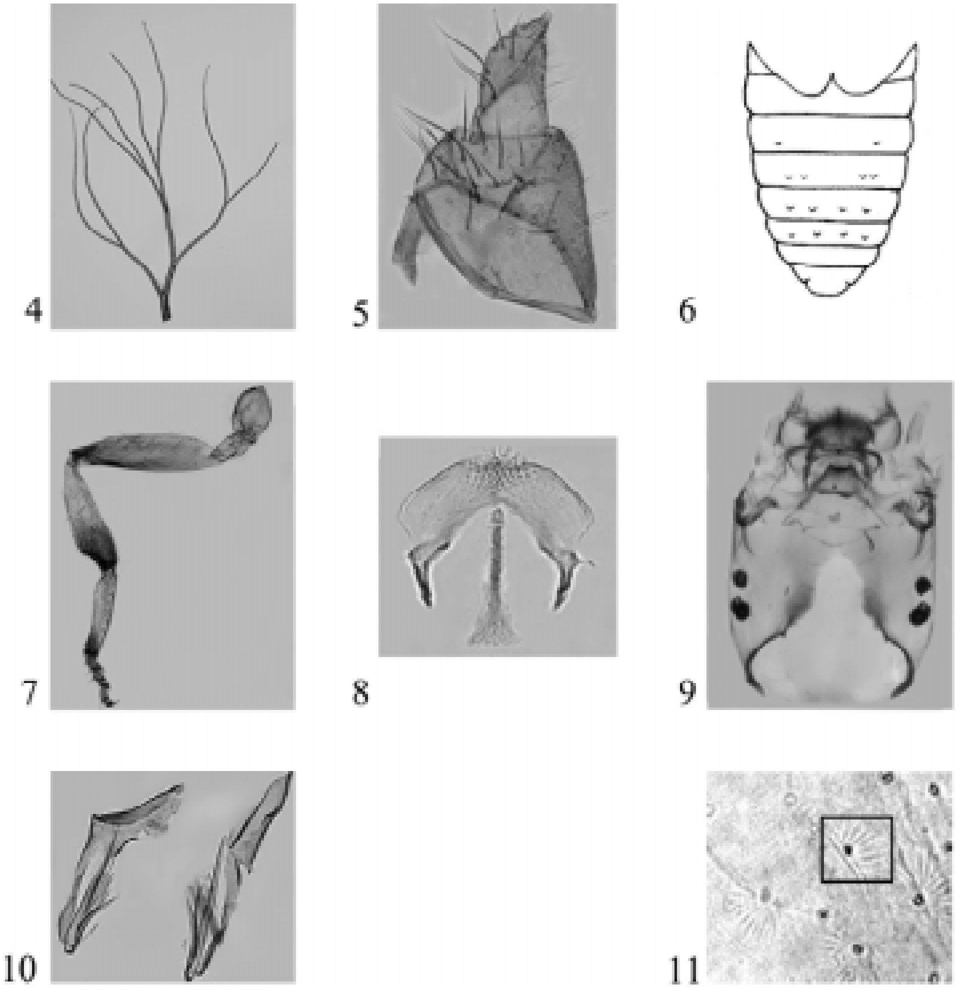
Adults: Golden scales spread evenly over scutum of ♂ and ♀ , male genitalia differences (Figs 5, 8 and10) and sub-basal dark band on the female hind tibia absent (Fig. 7).

Adult female.

General body colour silvery grey.

Head: Eye facets mostly silver but some black; frons greyish with golden scales; antennae brownish with basal segments paler; postcranial hairs golden.

Thorax: Grey when viewed with anterior or posterior light source; no apparent scutal pattern except evenly clothed with golden scales; pleural membrane and katipisternum pruinose silvery-grey with no scales; scutellum silvery-grey with golden scales and long black hairs; wing stem vein tufts dark; subcostal wing vein with hairs along proximal two-thirds; arculus medium brown; halteres with dark stem and beige to dark brown crown; legs all dark except the tibiae which are golden on the top half and the hind



Figs 4–11. *Simulium santomi* sp. n. 4. Pupal gill. 5. Gonocoxite and gonostyle. 6. Pupal ventral onchotaxy. 7. Female hind leg. 8. Ventral plate and median sclerite. 9. Larval postgenal cleft. 10. Parameres. 11. Larval abdominal setae.

basitarsi which are golden on the top two-thirds, hind tibia (Fig. 7) lacks sub-basal band present on female *S. alcocki*; claw curved with well developed basal tooth.

Abdomen: Basal scale of short golden hairs; abdominal tergites black, shiny distally, dorsally covered in golden scales, distally mostly with dark hairs, laterally covered with golden scales on 2–5.

Terminalia: Eighth sternite lightly sclerotised, covered in minute setae with groups of about 30 long thin setae on each side; gonopophyses well developed, membranous and covered in minute setae; paraprocts conical; genital fork with thin base and thin lateral arms with well formed anterior processes; spermatheca oval with no apparent external sculpturing or internal hairs; area of insertion of spermathecal duct membranous.

Adult male.

General body colour black.

Head: Upper eye facets reddish brown; lower eye facets grey to black, golden hairs along line where upper facets abut; postcranial hairs golden; antenna brownish.

Thorax: Scutum silvery when viewed with anterior light source, velvety black with posterior light source, and with no apparent pattern except evenly distributed golden scales; scutellum dark with golden scales and dark hairs; pleural membrane pruinose silver, no scale patch; katepisternum prinose brownish; legs all dark except tibiae and hind basitarsi which are both golden on the top half; wings stem vein tufts dark; arculus medium brown; subcostal vein bare; halteres entirely dark.

Abdomen: Basal fringe of long golden hairs; background colour velvety black; silver patches laterally on segments 2, 5 and 6; short dark hairs all over abdomen.

Terminalia: Style slightly tapered and in opposite orientation on coxite (Fig. 5) to that of *S. alcocki*; ventral plate broad, trilobed at base and indented at top (Fig. 8); median sclerite flat and strap-like and abruptly expanded apically (Fig. 8); paramere with multiple hooks (approximately 4–6) (Fig. 10).

Pupa.

Pupal length ventrally 1.90–2.60 mm (mean = 2.30, n =11). Gill length 2.05–2.50 mm (mean = 2.28, n =9). Cocoon length dorsally 2.00–2.70 mm (mean = 2.32, n =11).

Light brown slipper shaped cocoon with variable median anterior projection; cocoon slightly strengthened anteriorly and loosely woven with medium to large fibres; respiratory organ with 8 filaments, bifurcating from outside-in 2+((2+2)+2) (Fig. 4); gill filaments long and delicate with tips easily broken off, the lengths between the branches off the main stalk are usually longer than *S. alcocki*; cephalothorax with numerous dome-like tubercules and a few long single and bifid trichomes; abdomen dorsally with usual 4 hooks on each side of segments 3 and 4, whilst segments 7–9 each have a row of miniature spines; ventrally segment 4 with one hook on each side, segment 5 with pair of closely set hooks on each side and segments 6 and 7 with pair of more evenly set hooks on each side (Fig. 6).

Larva.

Length laterally 3.10–3.90 mm (mean = 3.56, n =13).

Head: Head capsule creamy yellow with very faint pigmentation and a negative head pattern, length 0.46–0.58 mm (mean = 0.53, n = 6), width 0.41–0.46 mm (mean = 0.43, n = 6); cephalic fan with 25–33 rays; postgenal cleft large, shaped like gothic arch (Fig. 9); ratio of postgenal cleft to postgenal bridge 2.0–2.4; hypostomium bearing 9 apical teeth with middle and corner teeth prominent; 4 hypostomial setae in each row parallel to margin of hypostomium; mandible as in *S. alcocki*.

Thorax: Speckled greenish-greyish; cuticle with minute broad fan-shaped setae and long single hairs; proleg circlet with about 27 rows of 2–6 hooks; pupal gill histoblast with 8 thin tightly coiled filaments.

Abdomen: Mottled greyish-brown dorsally, milky white ventro-apically; cuticle covered with minute fan-shaped setae and long single hairs (Fig. 11); rectal organ with 3–4 thick secondary lobules on each lobe; ventral papillae pointed; anal sclerite present; accessory sclerites present; posterior circlet with 60–70 rows of 2–12 hooks.

Type material: SÃO TOMÉ ISLAND: **Holotype** ♂ (pinned) with associated pinned pupal exuviae, abdomen and genitalia on slide, site 4, 10.x.1998, RJP (BMNH). **Paratypes**: 2 larvae, 1 pupa, site 3, 10.xi.1998, RJP (BMNH); 1♀ (pinned), 6 larvae, site 3, 10.xi.1998, RJP (ST&P); 1♂ (slide), 3♀ (slides), 10 larvae, site 4, 10.xi.1998. RJP (BMNH); 1♂ (slide), 3♀ (pinned), 3♀ (slides), 19 larvae, site 4, 10.xi.1998, RJP (ST&P); 20 larvae, site 5, 10.xi.1998, RJP (BMNH); 45 larvae, 3 pupae, site 5, 10.xi.1998, RJP (ST&P); 3 larvae, site 7, 12.xi.1998, RJP (BMNH); 1♀ (pinned), 1♀ (slide), 12 larvae, 2 pupae, site 8, 12.xi.1998, RJP (BMNH); 2♀ (pinned), 36 larvae, 6 pupae, site 8, 12.xi.1998, RJP (ST&P); 28 larvae, site 11, 11.xi.1998, RJP (ST&P); 1♂ (pinned, abdomen and genitalia on slide), 1♀ (pinned), 12 larvae, 2 pupae, site 12, 11.x.1998, RJP (BMNH); 1♂ (pinned), 3♀ (pinned), 14 larvae, 2 pupae, site 12, 11.x.1998, RJP (ST&P); 1 pupa, site 13, 13.xi.1998, RJP (BMNH); 6 larvae, site 14, 13.xi.1998, RJP (ST&P); 17 larvae, site 14, 13.xi.1998, RJP (ST&P); 6 larvae, site 15, 11.xi.1998, RJP (ST&P); 3 larvae, site 16, 11.xi.1998, RJP (BMNH).

Material seen from the Instituto de Higiene e Medicina Tropical (IHMT), Portugal (for full explanation of river sites and bioecological data see dos Santos Grácio 1999): SÃO TOMÉ ISLAND*:* 1 dissected pupal slide, no 129, site 9, 05.viii.1988, AJSG; 1 dissected pupal slide, no 230, site 36, 24.vii–07.viii.1988, AJSG; 2 dissected larval slides, no 467 & 468, 5 dissected pupal slides, no 96, 98, 99, 100 &135, site 39, 24.vii–07.viii.1988, AJSG.

Remarks: *Simulium santomi* constituted approximately 40 % of all specimens from São Tomé. It is clearly a member of the subgenus *Pomeroyellum* and all life stages key out as such following Crosskey (1969). With respect to species-groups, *S. santomi* keys out to the *alcocki* species-group following Freeman & de Meillon (1953), and clearly fits the *alcocki* species-group description of Crosskey (1969) better than any other species-group within *Pomeroyellum*.

Simulium (*Pomeroyellum*) *alcocki* Pomeroy, 1922

(Figs 12–20)

Simulium alcocki Pomeroy, 1922 (original description [Nigeria], ♂, ♀, pupa); Gibbins 1941 (*S. alcocki* variety *henrardi*); Roubaud & Grenier 1943 (larva); Freeman & de Meillon 1953 (♂, ♀, pupa); Crosskey 1960*a* (larva); Garms & Post 1967 (larval branched hairs, ♂ ventral plate); Crosskey & Howard 1997 (country distribution); dos Santos Grácio 1999 (first record São Tomé).

New material seen:

SÃO TOMÉ ISLAND: 22 larvae, 15 pupae, site 6, 12, xi.1998, RJP (BMNH); 70 larvae, 50 pupae, site 6, 12, xi.1998, RJP (ST&P); 1 ♂ (pinned), 1 ♂ (slide), 1 ♀ (pinned), 3 ♀ (slides), 11 larvae, 21 pupae, site 7, 12, xi.1998, RJP (BMNH); 3 ♂ (pinned), 2 ♀ (pinned), 38 larvae, 36 pupae, site 7, 12, xi.1998, RJP (ST&P); 27 larvae, site 8, 12.xi.1998, RJP (ST&P); 1 pupa, site 9, 11.xi.1998, RJP (ST&P).

Material seen from Natural History Museum (BMNH):

Paratypes: NIGERIA: reared adults 2♂2♀, BM 1921-148, Ibadan, 06.xii.1920, A. W. J. Pomeroy. **Other material**: CENTRAL AFRICAN REPUBLIC: 5 pupae, 2 larvae, Bangui, xii.1984–vi.1985, S. E. O. Meredith. DEMOCRATIC REPUBLIC OF CONGO:

4♀, BM 1951-347, Blukwa, 1951, A. Fain; slide of 8 filament pupal gills labelled *S. alcocki* variety *henrardi*; slide of 8 filament pupal gill labelled *S. alcocki* variety *henrardi*, Leo II Rhodiby, 6.xii.1934, C. Henrard. EQUATORIAL GUINEA: 2 pupae, Rio Sampaca, 8.iv.1998, P. J. McCall; 2 pupae, Rio Biolo/Timbabe, 8.iv.1998, P. J. McCall. GHANA: 1 pupa, BM 1949-525, M. H. Hughes; reared adults 2♂2♀, BM 1950-632, Yahala, small stream near Jo, 07.x.1950, L. Berner; 1♂, Senchi, vi.1955, M. Hughes. GUINEA: 6 pupae, 5 larvae, Saba. b. Santia, 8.iii.1963, R. Garms. NIGERIA: 10 pupae, 5 larvae, Abuja, 1959, R. W. Crosskey; 16 larvae, Abuja, 1959, R. W. Crosskey; 2 pupae, Vogu peak, 4500 ft, Northern Nigeria, 17.v.1969, H. Roberts. MALAWI: 1 pupa, Limbe, 14.viii.1952, L. Berner; 11 pupae, BM 1952-451, 11.viii.1952, L. Berner; 2 reared ♀, numbered 1738, Thyolo, R. C. Woods. RWANDA-BURUNDI (formerly Ruanda-Urundi): 1 reared 8 filament ♂, BM 1951-347, 1951, A. Fain. SIERRA LEONE: 3 pupae, 1926, B. Blacklock; 2♂, 2♀, BM 1925-564, xi.1925, B. Blacklock. SUDAN: 4 pupae, Khor Bangara, 5.x.1949, D. J. Lewis. TANZANIA: 1 pupa, Mahenge Mountains, 11.viii.1952, L. Berner. UGANDA: 2 pupae, 2 larvae, West Nile District, 20–21.x.1964, R. W. Crosskey.

Material seen from the Instituto de Higiene e Medicina Tropical (IHMT) (for full explanation of river sites and bioecological data see Santos Grácio 1999):

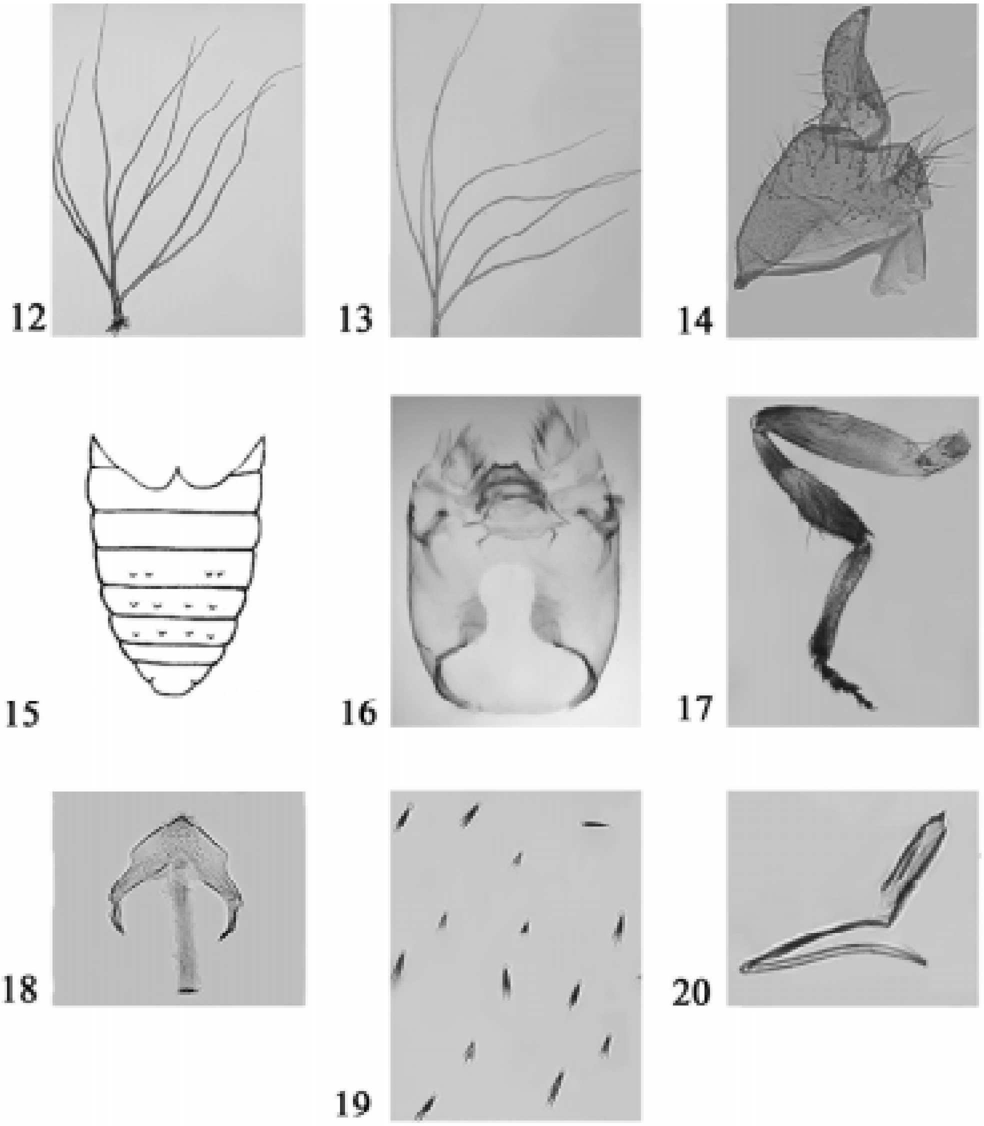
SÃO TOMÉ ISLAND*:* 1 larva on slide, no 545, 2 pupae on slides, no 215 & 216, site 23, 28, vii.1988, AJSG; 1 pupa on slide, no 207, site 28, 08.viii.1988, AJSG; 3 pupae on slides, no 89, 90 & 91, site 30, 30.vii.1988, AJSG.

Material seen from the Koninklijk Museum voor Midden-Afrika (MRAC):

DEMOCRATIC REPUBLIC OF CONGO: 1 pupa on slide, River Mampeza, Leopoldville; 5.xi.1942, Dr Henrard; 1 pupal skin on slide, labelled *S. alcocki* variety II, River Binga; 2 reared adult males, labelled Paratypes of *S.alcocki* variety *henrardi*, Leopoldville, no 208, 19.viii.1941, Dr Henrard; 1 reared adult female labelled 'Eg3 A' type II, River du Hazaret, Leopoldville, no 105, 19.iv.1941, Dr Henrard; 1 reared adult female, River du Hazaret, Leopoldville, no 105, 19.iv.1941, Dr Henrard.

Remarks: *Simulium alcocki* was not found in collections from Príncipe, but it made up approximately 30 % of samples from São Tomé, mostly in the northeast of the island. All life stages of *S. alcocki* examined were similar to descriptions in published records and museum material from East, Central and West Africa (including pupal gill variation).

The pupal gill branching pattern of *S. alcocki* collected from São Tomé island during the present survey was of two types. The first was the common seven filament type as recorded by Crosskey (1960*b*) from Nigeria and the Mount Cameroon area, and by Grenier *et al.* (1960) from across West Africa. The second had eight filaments (as illustrated by Freeman & de Meillon (1953) from the Sudan - see their figure 9m) and reported by dos Santos Grácio (1999) from São Tomé. In most places where it was found on São Tomé the seven filament form was sympatric with the eight filament form. A few specimens had a 'vestigial' eighth filament, and some others had seven filaments on one side and eight on the other. Examination of the external morphology of larvae, pupae and adults, and genital preparations from pupae and adults revealed no other differences between the seven and eight filament forms. The extra filament is clearly an example of intraspecific variation. Grenier & Rageau (1949) have also



Figs 12-20. *Simulium alcocki* Pomeroy. 12. Pupal gill of variety *henrardi*. 13. Typical pupal gill. 14. Gonocoxite and gonostyle. 15. Pupal ventral onchotaxy. 16. Larval postgenal cleft. 17. Female hind leg. 18. Ventral plate and median sclerite. 19. Larval abdominal setae. 20. Paramere.

described specimens with seven filaments on one side and eight on the other within populations from Cameroon.

The possession of eight gill filaments is the sole defining character mentioned by Gibbins (1941) in his description of *S. alcocki* variety *henrardi*. The holotype pupa of *Simulium alcocki* variety *henrardi*, described by Gibbins in 1941 from the Mampoza river at Leopoldville (now Kinshasa), could not be located. Gibbins (1941) also recorded larvae, pupae and adults of *S. alcocki* variety *henrardi* from two sites in Uganda, but this material was also not located during this study.

However, some material collected by Henrard was obtained from MRAC, including two pinned adult males labelled 'paratype' and determined as *S. alcocki* variety *henrardi* by Gibbins, two pinned adult females and two slides of pupal gills. The males were also labelled '8 filament', but on one the label had been later changed to '7 filament'. One of the pinned adult females was labelled *S. alcocki* type II and the other did not have a species label on it, but was identified as *S. alcocki*. An eight filament reared male adult which Fain collected in Rwanda-Burundi is present in BMNH. All external morphological features of these adults were consistent with those of typical *S. alcocki* (from published descriptions and specimens examined in BMNH).

It was not possible to view the genitalia because these were already missing from the '8 filament' male specimen labelled 'paratype' from MRAC. The first slide sent from MRAC was labelled as '*S. alcocki* variety *henrardi*'. The second slide was labelled '*S. alcocki* variety II'. Two slides of pupal gills labelled *S. alcocki* variety *henrardi* were also present at BMNH. These slides were assumed to be Gibbin's material because of his usual style of ringing his slide preparations with a black plastic material. The pupal gills on all these slides are identical to those of the eight filament *S. alcocki* present on São Tomé and different from the eight filament gill structure of *Simulium santomi* sp. n. described above.

Based on this evidence we have concluded that the seven filament and eight filament forms of *S. alcocki* on São Tomé are variations of the same species, and that the eight filament form is that described by Gibbins (1941) as *S. alcocki* variety *henrardi*, which is itself an example of intraspecific variation in the pupal gill, and not a subspecies. The name *henrardi* should henceforth be considered a junior synonym of *S. alcocki*.

Simulium (*Anasolen*) *dentulosum* Roubard, 1915

Simulium dentulosum Roubard, 1915 (original description [Uganda], ♀); Pomeroy 1916 (original description of *S. gilvipes* Pomeroy [Cameroon], synonym, ♂, ♀ , pupa); Freeman & de Meillon 1953 (♂, ♀ , pupa); Crosskey 1960*a* (larva); Crosskey & Howard 1997 (country distribution); dos Santos Grácio 1999 (first record São Tomé).

Material seen:

SÃO TOMÉ ISLAND: 27 larvae, site 1, 14.xi.1998, RJP (ST&P); 24 larvae, 4 pupae, site 2, 14.xi.1998, RJP (ST&P); 14 larvae, site 3, 10.xi.1998, RJP (ST&P); 2 pupae, site 5, 10.xi.1998, RJP (ST&P); 4 larvae, site 7, 12.xi.1998, RJP (ST&P); 12 larvae, 1 pupa, site 8, 12.ix.1998, RJP (ST&P); 10 larvae, site 10, 12.xi.1998, RJP (BMNH); 41 larvae, site 10, 12.xi.1998, RJP (ST&P); 2 larvae, site 11, 11.xi.1998, RJP (ST&P). PRÍNCIPE ISLAND: 10 larvae, 3 pupae, site 1, 18.xi.1998, RJP (BMNH); 33 larvae, 10 pupae, site 1, 18.xi.1998, RJP (ST&P); 2 larvae, 3 pupae, site 2, 18.xi.1998, RJP (ST&P); 1 larva, site 3, 17.xi.1998, RJP (ST&P); 33 larvae, 9 pupae, site 4, 17.xi.1998, RJP (BMNH); 84 larvae, 16 pupae, site 4, 17.xi.1998, RJP (ST&P); 11 larvae, site 5, 17.xi.1998, RJP (ST&P); 4 larvae, site 6, 16.xi.1998, RJP (ST&P); 10 larvae, site 7, 16.xi.1998, RJP (ST&P); 17 larvae, site 8, 17.xi.1998, RJP (ST&P); 25 larvae, 10 pupae, site 9, 19.xi.1998, RJP (ST&P); 15 larvae, 1 pupa, site 10, 19, xi.1998, RJP (ST&P); 11 larvae, 1 pupa, site 11, 19, xi.1998, RJP (BMNH); 29 larvae, 8 pupae, site 11, 19, xi.1998, RJP (ST&P); 33 larvae, 4 pupae, site 12, 19.xi.1998, RJP (ST&P); 18 larvae, site 13, 17.xi.1998, M. Pinheiro de Melo (BMNH).

Remarks: *Simulium dentulosum* was found at all altitudes in both large and small rocky cascading streams. It was the only species found on Príncipe, and dominated in the west of São Tomé island, where these sorts of streams are common.

DISCUSSION

The identification of larvae, pupae and neonate adults from 17 localities on the island of São Tomé showed *S. dentulosum* to be present in nine of the localities, *S. santomi* in eleven localities and *S. alcocki* in four localities. *Simulium santomi* was particularly abundant in the eastern part of the island, whereas *S. dentulosum* was dominant in the western part. In the north-eastern corner *S. alcocki* was also found. These results differ somewhat from those published by dos Santos Grácio (1988 1990 1999), in which *S. dentulosum* was the predominant species, while *S. alcocki* occurred in the central area of the island, (and *S. santomi* was not distinguished from *S. alcocki*). These differences could be the result of seasonal changes.

As is often the case for simuliids, differences in the form of the pupal gill were the first clues as to the presence of a new species here named *S. santomi* sp. n. However, the pupal gill branching pattern is also highly variable within some simuliid species, which can make taxonomic interpretation difficult. Nevertheless, the characteristic form of the pupal gill in *S. santomi* is consistently associated with a whole range of other characters in larvae, pupae and adults. The differences in male genitalia are particularly important in determining taxonomic status because such differences often occur between species. In insects it is generally argued that differences in genitalia between species contribute to genetic isolation by protecting against interspecific hybridisation through a lock and key analogy.

There are alternative possible evolutionary scenarios which might help to explain the occurrence of *S. santomi* on the island of São Tomé. One scenario involves an initial colonisation of São Tomé by *S. alcocki*, which then evolved (speciated) into *S. santomi* through either genetic drift (random genetic changes in small founder populations) or adaptation to the local environment (see Barton 1996), followed by a second colonisation by *S. alcocki* to give the current day situation. The other possible scenario involves a single colonisation of São Tomé by *S. alcocki* followed by sympatric speciation of *S. santomi*. Current models of sympatric speciation require microhabitat selection and specialisation by the incipient species with mate-selection within the microhabitat (Bush 1994). There are some differences in the geographical distribution of the two species on São Tomé, which may reflect differences in microhabitat requirements, but there is insufficient understanding of the ecologies of these species to decide whether this is likely to be significant.

There are no published records of onchocerciasis from the Republic of São Tomé and Príncipe, and the disease is probably not endemic. Unpublished observations also suggest that there are no Simuliidae on Annobón, the only island south of São Tomé (J. Mas *pers. comm.*). In contrast, onchocerciasis is widely known in southern Cameroon and on the island of Bioko, where it is transmitted by members of the *Simulium* (*Edwardsellum*) *damnosum* Theobald complex (Post *et al*. 2003). A vector elimination programme is currently being prepared for Bioko under the auspices of the Ministry of Health of the Republic of Equatorial Guinea, in collaboration with the World Health Organization African Programme for Onchocerciasis Control (WHO/APOC), and the

Spanish Co-operation. The drug ivermectin is currently being distributed as an ameliorating measure (McCall *et al.* 1998). The survey of São Tomé and Príncipe proved negative for *S. damnosum* s.l., confirming the results of dos Santos Grácio (1988, 1990, 1999) for São Tomé island. This has two important implications. Firstly, because the vector does not breed there, movement of infected people from Bioko or endemic areas of continental Africa (e.g. Angola, Democratic Republic of Congo, Gabon) will not result in onchocerciasis transmission and the development of a new focus in São Tomé and Príncipe. Secondly, the WHO/APOC vector elimination programme on Bioko cannot be jeopardised by immigration of potential vectors from São Tomé and Príncipe.

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